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Fibroblast Growth Factor 21, Adiponectin, and Irisin as Markers of Unfavorable Metabolic Features in 12-Year-Old Children

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Context: Among cytokines, fibroblast growth factor 21 (FGF21), adiponectin (Adn), and irisin have been considered potential biomarkers for insulin sensitivity (IS).

Objective: We evaluated whether serum FGF21, Adn, and irisin associate with markers of IS and serum lipids in 12-year-old children.

Design, Participants, and Main Outcome Measures: This cohort study included 192 12-year-old children (109 girls). Seventy-eight of them had been born appropriate for gestational age (AGA), 70 small for gestational age (SGA), and 44 from preeclamptic pregnancies (PREs) as AGA. Fasting serum FGF21, Adn, irisin, lipids, inflammatory markers, and IS markers were measured. Quantitative insulin sensitivity check index (QUICKI) was calculated.

Results: The means of serum FGF21, high molecular weight (HMW) Adn, and irisin did not differ between the sexes or between the SGA, AGA, and PRE children. In the whole study population, FGF21 associated positively with irisin and uric acid and negatively with leptin and high-density lipoprotein cholesterol (HDL-C). HMW Adn associated positively with total Adn, HDL-C, leptin, and SHBG. Apart from FGF21, irisin associated positively with insulin, high-sensitivity C-reactive protein, γ-glutamyltransferase, and triglycerides, and negatively with QUICKI, SHBG, and IGF binding protein-1. In multivariate regression analyses, irisin predicted lower IS and HMW Adn predicted higher HDL-C body mass index-independently, whereas FGF21 had no independent contribution to IS or lipid variables.

Conclusion: In 12-year-old children, serum irisin was associated with markers reflecting reduced IS. HMW Adn predicted HDL-C, whereas FGF21 did not contribute to IS or lipid parameters in multivariate regression analyses.

Freeform/Key Words: adipocytokines, myokines, cardiovascular disease risk, insulin resistance

Abbreviations: Adn, adiponectin; AGA, appropriate for gestational age; ANCOVA, analysis of covariance; AT, adipose tissue; B, breast development; BMI, body mass index; BMIadj, body mass index adjusted for sex and adult age; BP, blood pressure; CV, coefficient of variation; CVD, cardiovascular disease; FGF21, fibroblast growth factor 21; FNDC5, fibronectin type III domain-containing protein 5; G, genital development; GGT, γ-glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; HMW, high molecular weight; hs-CRP, high-sensitivity C-reactive protein; IGFBP-1, IGF binding protein-1; IS, insulin sensitivity; LDL-C, low-density lipoprotein cholesterol; PRE, preeclamptic pregnancy; QUICKI, quantitative insulin sensitivity check index; SGA, small for gestational age; TG, triglyceride; WHtR, waist-to-height ratio.

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Adipose tissue (AT) and skeletal muscles secrete cytokines, specifically adipokines and myokines that interact with each other. These cytokines are able to modulate energy homeostasis, adipogenesis, inflammation, endothelial function, glucose metabolism, and insulin sensitivity (IS), and thus body composition [1, 2]. Metabolic stress in white AT leads to dysregulated cytokine synthesis and secretion, which may contribute to obesity-associated metabolic, inflammatory, and cardiovascular comorbidities [1].

Fibroblast growth factor 21 (FGF21) is a cytokine produced primarily in the liver but also in other tissues such as skeletal muscle, white and brown AT, and pancreas [3, 4]. It belongs to the FGF superfamily, but unlike most of the FGFs it acts mainly in an endocrine manner [3]. To bind to its receptors and initiate signaling, FGF21 needs a cofactor, β-Klotho, expressed in a few metabolic tissues [3, 4]. According to animal studies, FGF21 has beneficial effects on IS, glucose, and lipid metabolism and energy homeostasis [3], and it regulates thermogenesis [5]. In humans, elevated FGF21 levels have been reported in insulin-resistant states and to independently predict metabolic syndrome and type 2 diabetes [6–8]. Furthermore, FGF21 has been associated with obesity already in childhood [9], but this finding is not consistent [10].

Adipocyte-produced adiponectin (Adn) [1, 11] mediates the beneficial effects of FGF21 on IS and energy metabolism in the skeletal muscle and liver [3]. Furthermore, Adn itself has anti-inflammatory, insulin sensitizing, energy expenditure increasing, and antiapoptotic properties [1, 11]. In clinical studies, circulating Adn correlated negatively with insulin resistance, visceral fat amount, type 2 diabetes, serum lipid levels, and blood pressure (BP) [1, 11]. Its low circulating concentrations predict type 2 diabetes, whereas its association with cardiovascular disease (CVD) is obscure [1]. The serum concentration of high molecular weight (HMW) Adn has been shown to correlate better with systemic IS than that of the low molecular weight isoform [1, 11].

The myokine irisin is a cleavage product of the fibronectin type III domain-containing protein 5 (FNDC5). Irisin is secreted mainly by muscle, but AT and other tissues secrete small amounts of it. In mice, irisin increases energy expenditure by stimulating the browning of white AT, and it improves glucose homeostasis and lipid profile [12]. In adults and children, elevated irisin concentrations have been observed in insulin resistance and obesity [12–16], but they decrease in type 2 diabetes [17]. However, the results in human studies have been inconsistent [12].

The aim of the current study was to determine whether serum FGF21, HMW Adn, and irisin concentrations associate with cardiometabolic risk markers, such as reduced IS or altered lipid profiles in 12-year-old children. Furthermore, we wanted to investigate whether they associate with low birth weight or exposure to maternal preeclampsia, which are considered to independently predispose to later metabolic disorders and CVD [18–20]. Finally, we sought to compare FGF21, HMW Adn, and irisin with other insulin resistance–related parameters in terms of detecting reduced IS, unfavorable lipid profiles, and elevated 24-hour ambulatory BP.

1. Materials and Methods

A. Definitions

Preeclampsia was defined as the development of hypertension and proteinuria (>300 mg of urinary protein in 24 hours) after 20 weeks' gestation [21]. Hypertension was defined as BP >140/90 mm Hg or a rise of ≥30/15 mm Hg from the baseline level, confirmed by two measurements ≥6 hours apart. *Full-term* indicates babies born at or after week 37 and before the 42nd week of gestation, and *preterm* indicates babies born before the 37th week of gestation (calculated from the beginning of the last menstruation). Small for gestational age (SGA) was defined as birth weight and/or length >2 SD scores below the respective mean for the gestational age and sex. Appropriate for gestational age (AGA) was defined as birth weight and birth length equal to or above −2 SD scores and equal to or below +2 SD scores of the respective means for gestational age and sex [22].

B. Subjects

The study population consisted of a cohort of 192 12-year-old children who originally were recruited to a study investigating the metabolic consequences of either being born SGA or being born after a preeclamptic pregnancy (PRE). Of this cohort 109 were girls, 70 were born SGA, and 44 were born after a PRE as AGA. The median of the gestational ages was 38.0 weeks (range 28 to 42 weeks). Extremely preterm children born before gestational week 28 were excluded from the study. None of the participating children was exposed to exogenous glucocorticoids prenatally. All children were born at Kuopio University Hospital during a 22-month period between 1984 and 1986. The study protocol was approved by the Research Ethics Committee of Kuopio University Hospital. Informed written consent was obtained from the child and the parents.

C. Methods

Perinatal data, anthropometric measures, and ambulatory BP values at 12 years age have been described previously for the SGA [23, 24] and PRE children [25, 26]. Pubertal development was classified as Tanner stages according to breast development (B) in girls and genital development (G) in boys. Body mass index (BMI; calculated as weight in kilograms divided by height in meters squared) and sex- and adult age–adjusted BMI (BMIadj; corresponding to the BMI values at the age of 18 years) [27] were calculated. Waist-to-height ratio (WHtR) was calculated by dividing waist circumference (in centimeters) by height (in centimeters). Perinatal characteristics, anthropometric measures, and pubertal development at the age of 12 years in the whole study population are presented in Table 1.

C-1. Laboratory methods

Blood samples were taken in the morning, between 0900 and 1000 hours, after an overnight fast. An IV cannula was placed in the antecubital vein for blood sampling. After the child had rested for 1 hour in a recumbent position, blood samples were drawn through the cannula. Serum specimens were immediately frozen and stored at −70°C until analyzed.

Serum irisin concentrations were measured with ELISA kits (#EK-067-52, Phoenix Pharmaceuticals Inc, Burlingame, CA) [28]. The detection range of the assay was 0.066 to 1024 ng/mL, and the intra-assay and interassay coefficients of variation (CVs) were <6% and <10%, respectively. Serum FGF21 concentrations were measured with a highly specific ELISA kit (BioVendor, Brno, Czech Republic) [29], and the respective CVs were 2.0% and 3.3%. Serum total Adn and HMW Adn were measured by ELISA (Quantikine DRP300, R&D Systems, Inc., Minneapolis, MN; EZH MWA-64K, Merck Millipore, Darmstadt, Germany) [30, 31]; the intra-assay and interassay CVs for total Adn were <4.7% and <6.9%, and those for HMW Adn were <8.8% and 6.1%. Serum insulin concentrations were determined by RIA (Phadeseph Insulin RIA, Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). The intra-assay and interassay CVs for insulin were 5.3% and 7.6%, respectively. Blood glucose concentrations were analyzed by a glucose oxidase method (Enzyme Electrode, Nova Biomedical, Waltham, MA), and the respective CVs were 3% and 5%. Serum high-density lipoprotein cholesterol (HDL-C) and triglycerides (TGs) were measured enzymatically by an automatic photometric method (Roche Molecular Biochemicals, Mannheim, Germany), and the interassay CVs were 4.1% at 0.84 mM and 3.8% at 1.69 mM, and 3.2% at 1.23 mM and 1.6% at 2.45 mM, respectively. Low-density lipoprotein cholesterol (LDL-C) concentrations were calculated by the Friedewald-Fredrickson formula [LDL-C = total cholesterol – (HDL-C + TG/2.2)]. Serum IGF-1 and IGF binding protein-1 (IGFBP-1) concentrations were analyzed by ELISA (DSL-10-5600 Active IGF-I ELISA; DSL-10-7800 Active Total IGFBP-1 ELISA, both from Diagnostic Systems Laboratories, Inc., Webster, TX) [32, 33]. The intra-assay CV for IGF-1 was 6.5%, and the interassay CV was 6.4%, as reported by the manufacturer; for IGFBP-1 the respective CVs were 2.5% and 6.8%. Serum SHBG was measured by the AutoDELFIA SHBG time-resolved fluoroimmunoassay method (Perkin Elmer Life Sciences Wallac, Turku, Finland). The intra-

Table 1. Anthropometric Characteristics and Biochemical Parameters for the Whole Study Population and for Girls and Boys Separately

Variable	All (n = 192)	Girls (n = 109)	Boys (n = 83)	P^a	P^b
At birth					
Gestational age, wk[c]	37.5 (37.0, 38.0)	37.9 (37.3, 38.6)	37.0 (36.3, 37.8)	0.069	
Weight, g	2769 (2662, 2877)	2736 (2586, 2886)	2813 (2659, 2967)	0.482	
Weight, SDS	−1.14 (−1.33, −0.95)	−1.27 (−1.55, −1.00)	−0.97 (−1.23, −0.71)	0.111	
Length, cm	47.3 (46.7, 47.8)	47.0 (46.3, 47.8)	47.6 (46.8, 48.4)	0.260	
Length, SDS	−0.88 (−1.09, −0.67)	−1.02 (−1.31, −0.74)	−0.69 (−1.00, −0.38)	0.117	
At the age of 12 y					
Age, yr[c]	12.25 (12.23, 12.27)	12.25 (12.22, 12.29)	12.25 (12.21, 12.28)	0.868	
Weight, kg[c]	43.00 (41.60, 44.44)	43.14 (41.26, 45.09)	42.81 (40.70, 45.03)	0.816	
BMIadj, kg/m^2[c]	21.15 (20.63, 21.68)	20.65 (20.02, 21.31)	21.81 (20.96, 22.70)	**0.028**	**0.001**[d]
Height, cm	153.2 (152.1, 154.2)	153.9 (152.4, 155.4)	152.2 (150.6, 153.8)	0.119	
Height, SDS	0.26 (0.12, 0.41)	0.16 (-0.04, 0.36)	0.40 (0.18, 0.61)	0.116	
WHtR	0.43 (0.42, 0.44)	0.42 (0.41, 0.43)	0.44 (0.43, 0.45)	**0.013**	**0.012**[d]
Pubertal development,[e] early/late stage, n	107/85	38/71	69/14	**<0.001**[f]	
Pubertal development				**<0.001**[f]	
Tanner B/G stage 1, n	40	14	26		
Tanner B/G stage 2, n	67	24	43		
Tanner B/G stage 3, n	50	38	12		
Tanner B/G stage 4, n	27	25	2		
Tanner B/G stage, 5 n	8	8	0		
S-FGF-21, ng/L [c]	95.7 (82.4, 111.2)	109.0 (89.2, 133.1)	80.8 (64.4, 101.3)	0.051	0.253
S-Adn, mg/L[c]	9.4 (8.7, 10.2)	9.8 (8.9, 10.8)	8.9 (7.7, 10.2)	0.375	0.150
S-HMW Adn, mg/L[c]	4.5 (4.1, 4.9)	4.5 (4.1, 5.0)	4.4 (3.7, 5.1)	1.000	0.612
S-Irisin, μg/L[c]	135.4 (128.0, 143.3)	132.5 (123.6, 142.0)	139.4 (126.9, 153.1)	0.378	0.458
S-Insulin, mU/L[c]	9.4 (8.9, 9.9)	10.3 (9.5, 11.1)	8.3 (7.7, 9.0)	**<0.001**	**<0.001**
B-Glucose, mmol/L	4.3 (4.3, 4.4)	4.3 (4.2, 4.3)	4.4 (4.3, 4.5)	**0.009**	**0.025**
QUICKI	0.351 (0.348, 0.354)	0.347 (0.342, 0.352)	0.356 (0.351, 0.361)	**0.007**	**0.005**
S-IGFBP-1, μg/L[c]	56.4 (51.8, 61.4)	51.6 (45.9, 58.0)	63.4 (56.0, 71.7)	**0.017**	**0.007**
S-IGF-1, μg/L	314.3 (296.3, 332.4)	360.5 (338.2, 382.7)	253.8 (228.9, 278.6)	**<0.001**	**<0.001**
S-SHBG, nmol/L	73.5 (68.9, 78.2)	69.6 (63.7, 75.5)	78.7 (71.3, 86.2)	0.060	**0.032**
S-Leptin, μg/L[c]	8.6 (7.3, 10.1)	11.2 (9.2, 13.6)	6.1 (4.7, 7.8)	**<0.001**	**<0.001**
S-GGT, U/L[c]	15.9 (15.5, 16.3) (n = 191)	15.4 (15.0, 15.8) (n = 108)	16.6 (15.9, 17.3)	**0.004**	0.082
S-Uric acid, μmol/L	276.7 (268.0, 285.4) (n = 191)	274.1 (262.7, 285.5) (n = 108)	280.1 (266.5, 293.7)	0.503	**0.027**
S-Triglycerides, mmol/L[c]	0.85 (0.06, 0.90)	0.90 (0.84, 0.98)	0.78 (0.72, 0.85)	**0.011**	**0.002**
S-HDL cholesterol, mmol/L	1.32 (1.28, 1.36)	1.30 (1.25, 1.35)	1.35 (1.29, 1.41)	0.223	0.904
S-LDL cholesterol, mmol/L	2.76 (2.66, 2.86)	2.79 (2.67, 2.91)	2.72 (2.56, 2.89)	0.410	0.054
S-hs-CRP, mg/L[c]	0.25 (0.20, 0.30) (n = 191)	0.21 (0.17, 0.27) (n = 108)	0.30 (0.22, 0.41) (n = 83)	0.126	0.376
24-h systolic BP, mm Hg	117 (116, 118) (n = 180)	116 (115, 118) (n = 100)	117 (116, 119) (n = 100)	0.354[g]	0.272[h]
24-h diastolic BP, mm Hg	68 (68, 69) (n = 180)	68 (67, 69) (n = 100)	69 (68, 70) (n =80)	0.380[g]	0.239[h]

The means (95% CIs) are presented. Significant P - values are bold.
Abbreviations: B, blood; S, serum; SDS, SD score.
[a]Independent-samples t test for the differences between the girls and boys.
[b]Comparison by ANCOVA adjusted for BMIadj and pubertal developmental stage (G/B 1 to 5).
[c]Geometric means (95% CIs) are presented for the variables with skew distributions.
[d]Comparison by ANCOVA adjusted for pubertal developmental stage (G/B 1 to 5).
[e]Early stage of puberty, breast or genital scores 1 to 2; late stage of puberty, breast or genital scores 3 to 5.
[f]Chi-square test for differences between girls and boys.
[g]Comparison by ANCOVA adjusted for BMIadj and height (SDS).
[h]Comparison by ANCOVA adjusted for BMIadj, pubertal developmental stage (G/B 1 to 5) and height (SDS).

assay and interassay CVs were 4.0% and 2.6%, respectively. Serum leptin concentrations were analyzed by ELISA (Quantikine DLP00, R&D Systems, Inc.) [34], with its intra-assay and interassay CVs 3.2% and 3.5%. Serum high-sensitivity C-reactive protein (hs-CRP) concentrations were determined by a photometric immunoturbidimetric method (Konelab 20XT Clinical Chemistry Analyzer, Konelab, Thermo Fisher Scientific, Vantaa, Finland), and the interassay CVs were 5.0% at 0.78 mg/L and 2.3% at 2.55 mg/L. Serum γ-glutamyl-transferase (GGT) and uric acid were measured by enzymatic photometric tests (Konelab 20 XT, Clinical Chemistry Analyzer, Thermo Fisher Scientific). The intra-assay CV for GGT was 3.3%, and the interassay CV was <2.4%; for uric acid the respective CVs were 4.0% and 2.9%. Quantitative insulin sensitivity check index (QUICKI) was calculated as 1/[log (fasting insulin, μU/mL) + log (fasting glucose, mg/dL)] [35].

D. Statistical Analyses

Data were analyzed with the statistical program SPSS for Macintosh, version 24.0 (SPSS, IBM Corp., Armonk, NY). All continuous variables were examined for normality with the Kolmogorov-Smirnov test. Skewed data were either logarithmically or square root transformed before testing. Independent samples *t* test, univariate ANOVA, or analysis of covariance (ANCOVA) were used for comparisons between groups. Sidak correction was used for *post hoc* tests. Linear regression analyses were used for estimating the associations between FGF21, HMW Adn, and irisin concentrations with cardiometabolic variables; standardized β-values are reported in these analyses. Multivariate regression analyses were performed to evaluate the independent contributions of measured cytokines, IS markers, and BMIadj to IS, lipid variables, and ambulatory BP values. $P < 0.05$ was accepted as significant in all analyses.

2. Results

A. Sex-Specific Anthropometric and Biochemical Characteristics of the Study Population

At the age of 12 years, the boys had higher WHtR and BMIadj than the girls (Table 1). Pubertal development was more advanced in the girls than boys: 87% of girls and 69% of boys had pubertal signs (Tanner stage B/G ≥2) (Table 1). The SGA-born children were shorter compared with the AGA-born and PRE children in terms of height SD scores (SGA vs AGA −0.19 and 0.63, $P < 0.001$; and SGA vs PRE −0.19 and 0.33, $P = 0.016$, respectively, by Sidak-adjusted ANOVA) and leaner than the AGA-born children measured by BMIadj (20.44 and 22.14 kg/m^2, $P = 0.020$, by Sidak-adjusted ANOVA). Pubertal development did not differ between the SGA, AGA, and PRE subgroups or between the preterm and full-term subjects (data not shown).

At the age of 12 years, the mean concentrations of serum FGF21 tended to be higher in the girls compared with the boys ($P = 0.051$). However, further adjustment for pubertal developmental stage and BMIadj attenuated the significance (Table 1). Serum total and HMW Adn and irisin concentrations did not differ between the sexes (Table 1). The girls had significantly higher serum IGF-1, insulin, leptin, and TG concentrations and lower QUICKI, blood glucose, and serum IGFBP-1 concentrations compared with the boys (Table 1). The sex difference in GGT was attenuated after adjustment for pubertal developmental stage and BMIadj (Table 1). SHBG and uric acid were significantly lower in the girls, when adjusted for pubertal developmental stage and BMIadj (Table 1). Pubertal developmental stage had no effect on serum FGF21 or irisin concentrations, when the sexes were analyzed separately (girls, $P = 0.934$ for FGF21 and $P = 0.305$ for irisin; boys, $P = 0.628$ for FGF21 and $P = 0.226$ for irisin, by Sidak-adjusted ANOVA), in the whole study population ($P = 0.447$ and $P = 0.176$, respectively, by Sidak-adjusted ANOVA), or between the sexes within the same Tanner stage (data not shown). However, the boys had lower HMW Adn concentrations in Tanner stage G3 than in stages G1 or G2 (G3 1.67 mg/L vs G1 2.33 mg/L, $P = 0.031$; and G3 1.67 mg/L vs G2 2.29 mg/L, $P = 0.029$, by Sidak-adjusted ANOVA) and lower than the girls in Tanner stage B3 (1.67 vs 2.18 mg/L, $P = 0.021$, respectively, by independent samples *t* test). The concentrations of serum FGF21, HMW Adn, irisin, GGT, IGF-1, IS markers (QUICKI, IGFBP-1, SHBG), or lipids did not differ between the AGA, SGA, and PRE subgroups or between the preterm and full-term subjects (data not shown).

B. Factors Associated With Serum FGF21, HMW Adn, and Irisin Concentrations

In the whole study population, FGF21 had a positive association with irisin and uric acid and a negative association with HDL-C in linear regression analyses adjusted for sex and pubertal developmental stage, even after further adjustment for BMIadj (Table 2). Furthermore, when adjusted for BMIadj, a negative association between FGF21 and leptin turned significant (Table 2). The positive association between FGF21 and irisin remained significant in the boys (n = 83, $\beta = 0.417$, $P < 0.001$ in analysis adjusted for both pubertal developmental stage and BMIadj). Moreover, FGF21 had a positive association with IGF-1 in

Table 2. Associations of Serum FGF21, HMW Adn, and Irisin Concentrations With Metabolic and Anthropometric Variables in 12-Year-Old Children

		FGF21				HMW Adn				Irisin			
		All				All				All			
Variable	**n**	β^a	***P***	β^b	***P***	β^a	***P***	β^b	***P***	β^a	***P***	β^b	***P***
FGF21	192	—	—	—	—	−0.036	NS	−0.027	NS	0.237	**0.001**	0.234	**0.001**
Adn	192	−0.083	NS	−0.081	NS	0.842	**<0.001**	0.829	**<0.001**	0.059	NS	0.070	NS
HMW Adn	192	−0.036	NS	−0.029	NS	—	—	—	—	0.045	NS	0.060	NS
Irisin	192	0.239	**0.001**	0.236	**0.001**	0.045	NS	0.057	NS	—	—	—	—
Insulin	192	0.030	NS	0.035	NS	−0.134	NS	−0.031	NS	0.278	**<0.001**	0.344	**<0.001**
Glucose	192	−0.029	NS	−0.029	NS	−0.098	NS	−0.078	NS	−0.001	NS	−0.002	NS
QUICKI	192	−0.020	NS	−0.022	NS	0.148	**0.048**	0.059	NS	−0.244	**0.001**	−0.290	**0.001**
IGFBP-1	192	0.055	NS	0.071	NS	0.212	**0.005**	0.161	0.071	−0.169	**0.026**	−0.211	**0.020**
IGF-1	192	0.014	NS	0.139	NS	−0.123	NS	−0.062	NS	0.136	NS	0.131	NS
SHBG	192	−0.027	NS	−0.031	NS	0.254	**<0.001**	0.190	**0.038**	−0.161	**0.033**	−0.203	**0.031**
Leptin	192	−0.116	NS	−0.283	**0.024**	0.022	NS	0.448	**<0.001**	0.035	NS	0.008	NS
GGT	191	0.085	NS	0.081	NS	−0.169	**0.023**	−0.116	NS	0.227	**0.002**	0.228	**0.003**
TG	192	0.091	NS	0.091	NS	−0.141	0.055	−0.082	NS	0.158	**0.031**	0.157	**0.041**
HDL-C	192	−1.493	**0.048**	−1.603	**0.043**	3.967	**<0.001**	3.641	**<0.001**	−0.268	NS	−0.144	NS
LDL-C	192	−0.083	NS	−0.089	NS	0.121	NS	0.135	0.062	0.040	NS	0.065	NS
Uric acid	191	0.197	**0.015**	0.181	**0.021**	0.002	NS	0.041	NS	−0.012	NS	−0.024	NS
hs-CRP	191	0.029	NS	0.036	NS	−0.081	NS	−0.011	NS	0.214	**0.004**	0.232	**0.003**
24-h systolic BP[c]	180	0.034	NS	0.009	NS	−0.076	NS	0.033	NS	0.037	NS	0.008	NS
24-h diastolic BP[c]	180	0.041	NS	0.009	NS	−0.026	NS	0.041	NS	0.129	NS	0.113	NS
BMIadj	192	−0.003	NS	—	—	−0.191	**0.013**	—	—	0.043	NS	—	—
WHtR	192	−0.034	NS	—	—	−0.179	**0.017**	—	—	0.011	NS	—	—
BW, SDS	192	−0.057	NS	—	—	−0.152	**0.042**	—	—	−0.020	NS	—	—
PRE	192	−0.120	NS	—	—	0.065	NS	—	—	−0.105	NS	—	—

Skewed variables were logarithmically or square root–transformed before testing. Standardized β-values are reported. Significant P - values are bold.
Abbreviations: BW, birth weight; NS, not significant; SDS, SD score.
[a]Linear regression analysis adjusted for sex and pubertal developmental stage (G/B 1 to 5).
[b]Linear regression analysis adjusted for sex, pubertal developmental stage (G/B 1 to 5), birth weight SDS, maternal PRE history, and BMIadj.
[c]Further adjusted for height SDS.

boys (n = 83, $\beta = 0.349$, $P = 0.014$) in BMIadj-adjusted analyses. FGF21 concentrations had no association with birth weight or preeclamptic history in linear regression analyses (Table 1).

We found positive associations between serum HMW Adn and QUICKI, IGFBP-1, SHBG, total Adn, and HDL-C and negative associations with serum GGT, BMIadj, and WHtR (linear regression analyses adjusted for sex and pubertal developmental stage) (Table 2). The associations between HMW Adn and QUICKI, IGFBP-1, and GGT were attenuated after BMIadj adjustment (Table 2). In contrast, the positive association with leptin turned significant in BMIadj-adjusted analyses (Table 2). The strong positive association between HMW Adn and HDL-C remained in the boys (n = 83, $\beta = 5.990$, $P < 0.001$), whereas it was attenuated in the girls after BMIadj adjustment (n = 109, $\beta = 1.942$, $P = 0.056$). In boys, HMW Adn had a negative association with TG and a positive association with leptin in BMIadj-adjusted analyses (n = 83; $\beta = -0.315$, $P = 0.011$; $\beta = 0.532$, $P = 0.003$, respectively). Furthermore, in boys BMIadj-dependent associations were found between HMW Adn and IGFBP-1, IGF-1, and SHBG (n = 83; $\beta = 0.241$, $P = 0.046$; $\beta = -0.292$, $P = 0.046$; $\beta = 0.273$, $P = 0.027$, respectively). In girls, HWM Adn associated positively with SHBG in linear regression analysis adjusted for pubertal developmental stage (n = 109, $\beta = 0.220$, $P = 0.021$). Finally, low birth weight (SD score) predicted independently low HMW Adn after adjustment for sex, pubertal developmental stage, and BMIadj in linear regression analysis (Table 2).

In the whole study population, serum irisin had positive associations with serum FGF21, insulin, GGT, hs-CRP, and TG and negative associations with QUICKI, IGFBP-1, and SHBG (linear regression analyses adjusted for sex and pubertal developmental stage) (Table 2). All these associations remained significant after further adjustment for BMIadj. In subgroup analyses, the associations between irisin and insulin, and irisin and QUICKI remained significant even after BMIadj adjustment in both girls (n = 109; $\beta = 0.325$, $P = 0.002$; $\beta = -0.272$, $P = 0.007$, respectively) and boys (n = 83; $\beta = 0.370$, $P = 0.018$; $\beta = -0.302$, $P = 0.041$, respectively). In girls, positive associations between serum irisin and GGT (n = 108, $\beta = 0.256$, $P = 0.025$), TGs (n = 109, $\beta = 0.210$, $P = 0.020$), and hs-CRP (n = 108, $\beta = 0.257$, $P = 0.018$) were found even after adjustments for BMIadj. In contrast, in boys BMIadj-independent associations were found between serum irisin and FGF21 (n = 83, $\beta = 0.482$, $P < 0.001$), IGFBP-1 (n = 83, $\beta = -0.312$, $P = 0.035$), SHBG (n = 83, $\beta = -0.372$, $P = 0.023$), and 24-hour diastolic BP (n = 80, $\beta = 0.283$, $P = 0.021$). In linear regression analyses, birth weight or preeclamptic history had no association with irisin concentrations (Table 2).

Associations of serum FGF21, HMW Adn, and irisin with metabolic and anthropometric variables in the SGA, AGA, and PRE subgroups are shown in an online repository [36]. Some of the unfavorable associations tended to be stronger in the SGA than AGA or PRE subgroups.

C. FGF21, HMW Adn, and Irisin as Markers of Reduced IS and Unfavorable Lipid Profile

Multivariate linear regression analyses were performed to estimate independent contributions of FGF21, HMW Adn, irisin, and other insulin resistance–related markers to cardiometabolic risk factors (estimated by QUICKI, 24-hour ambulatory systolic BP, and serum TG and HDL-C concentrations). In analyses adjusted simultaneously for both sex and pubertal stage, higher serum irisin and lower IGFBP-1 predicted lower IS independently of BMIadj. In addition, low birth weight had an independent contribution to low IS (Table 3). Higher serum HMW Adn was a positive predictor for HDL-C (Table 3). Low birth weight (SD score), maternal preeclampsia, and lower IGFBP-1 associated independently with higher 24-hour ambulatory systolic BP mean (Table 3), whereas FGF21, HMW Adn, or irisin had no independent contribution. The contribution of BMIadj was significant in all analyses with the exception of TG levels (Table 3).

3. Discussion

The current study revealed that serum irisin could predict reduced IS independently of BMIadj. In linear regression analyses, irisin was associated independently of BMIadj, sex, and pubertal

developmental stage with several markers reflecting reduced IS (low QUICKI, IGFBP-1, and SHBG). Serum HMW Adn was a positive predictor for HDL-C, whereas its associations with IS markers were BMIadj dependent. FGF21 had no independent effect on IS or lipid variables in multivariate regression analyses. Low birth weight was an independent contributor to lower IS and higher 24-hour ambulatory systolic BP values. Minor sex differences in the associations were found. Moreover, some of the unfavorable associations tended to be stronger in the children born SGA than in those born AGA or from preeclamptic pregnancies.

FGF21 inhibits lipolysis, induces browning of white AT, promotes pancreatic β-cell function and survival, and protects against insulin resistance and obesity [3–5]. In clinical studies, recombinant FGF21 has been shown to improve dyslipidemia, decrease insulin levels, and lower body weight, whereas no glucose-lowering effects have been found [4]. However, obesity has been proposed to be an FGF21-resistant state [6] and FGF21 to be a marker of adverse metabolic profiles in adults [8, 37]. Studies conducted in children or adolescents are scarce, with inconsistent results. Positive associations have been reported between FGF21 and BMI [9] or weight SD score [38]; however, the majority of the study subjects in these reports have been overweight or obese. Contrary to these studies, Li *et al.* [10] demonstrated recently in a large Chinese study that serum FGF21 was negatively associated with obesity. Finally, Hanks *et al.* [39] reported no association of FGF21 with weight parameters, which is consistent with our results based on mainly normal-weight 12-year-old children. Furthermore, the reported associations of FGF21 with insulin levels or insulin resistance indices have been either positive [38], negative [10], or insignificant [9], the latest in accordance with our findings. In our study, serum FGF21 associated negatively with HDL-C and leptin in BMIadj-adjusted linear regression analyses. Previously reported correlations of FGF21 with HDL-C have been positive [10], and those with leptin have been inconsistent [9, 10]. In normoglycemic and type 2 diabetic adults, high serum FGF21 levels were associated with an adverse lipid profile [6, 7]. However, null results have also been reported in these association studies [8]. Furthermore, we found no association of FGF21 with Adn, although FGF21 increases Adn expression and its serum levels [3]. In line with our findings, a study of overweight and obese children and adolescents found no association between FGF21 and Adn [38], whereas Li *et al.* [10] reported a positive correlation between these parameters. The discrepancies may be explained by differences in the sample sizes and metabolic status of the participants or by variable controlling of confounding factors.

In addition to adipocytes, Adn is expressed in many other cell types, including skeletal and cardiac myocytes and epithelial cells [11]. It not only regulates IS and energy homeostasis [1] but also improves lipid profile [11]. In adults, serum Adn levels decrease in obesity, type 2 diabetes, metabolic syndrome, and CVD [1]. HMW multimer is considered the most active Adn isoform [1, 11], and downregulation of HMW Adn has been shown to reflect vascular and metabolic abnormalities better than that of total Adn [40, 41]. Furthermore, low HMW Adn levels have been linked to obesity, especially abdominal obesity, already in childhood [40, 42, 43] and adolescence [41]. However, in accordance with our results, the association between HMW Adn and insulin resistance can be explained by adiposity [42]. In line with our results, BMI-independent positive associations between HMW or total Adn and HDL-C have been reported in adolescents [44] and adults [37]. Furthermore, in our SGA subgroup, the BMI-independent negative association of HMW Adn with TG was significant similarly to several other studies with children and adolescents [40, 41, 44].

Irisin was discovered primarily as an exercise-regulated myokine that induces browning of white AT [12, 45]. Its physiologic significance in humans is debated, and the reported findings are controversial. Furthermore, in humans, circulating irisin concentrations have been observed to differ greatly, and the validity of different assays has been questioned [12]. In some human studies with few participants, FNDC5 and irisin levels have been reported to increase after exercise, but this finding has not been confirmed by others [reviewed in 12]. Although in subjects with obesity FNDC5 expression in adipocytes and consequently irisin secretion is lower [12, 17], the concentrations of serum irisin may be similar or higher than in normal-weight subjects because of increased fat mass [12]. AT has been hypothesized to secrete irisin in

Table 3. Biochemical and Anthropometric Factors Predicting Cardiovascular Risk Markers in 12-Year-Old Children

Dependent Variable	Covariates	Independent Variable	r^2	β	P	n
QUICKI	Sex, pubertal stage	FGF21	0.504	−0.018	0.753	191
		HMW Adn		−0.020	0.727	
		Irisin		−0.125	**0.030**	
		IGFBP-1		0.457	**<0.001**	
		SHBG		0.102	0.169	
		GGT		−0.076	0.192	
		Uric acid		0.019	0.152	
		BMIadj		−0.183	**0.022**	
		Preeclampsia		0.001	0.597	
		Birth weight, SDS		0.002	**0.026**	
HDL-C	Sex, pubertal stage	FGF21	0.288	−0.122	0.073	191
		HMW Adn		0.343	**<0.001**	
		Irisin		−0.018	0.711	
		IGFBP-1		−0.002	0.716	
		SHBG		0.004	0.471	
		GGT		0.145	0.217	
		Uric acid		−0.068	0.481	
		BMIadj		−0.065	**0.022**	
		Preeclampsia		0.008	0.651	
		Birth weight, SDS		0.002	0.716	
Triglycerides	Sex, pubertal stage	FGF21	0.183	0.085	0.243	191
		HMW Adn		−0.030	0.683	
		Irisin		0.066	0.385	
		IGFBP-1		−0.009	0.232	
		SHBG		−0.015	0.079	
		GGT		0.151	0.403	
		Uric acid		−0.283	0.057	
		BMIadj		0.050	0.258	
		Preeclampsia		−0.002	0.942	
		Birth weight, SDS		−0.017	0.076	
24-h systolic BP	Sex, pubertal stage	FGF21	0.238	−0.002	0.970	180
		HMW Adn		0.030	0.467	
		Irisin		−0.107	0.481	
		IGFBP-1		−0.034	**0.020**	
		SHBG		0.004	0.802	
		GGT		0.603	0.097	
		Uric acid		0.210	0.477	
		BMIadj		0.192	**0.031**	
		Preeclampsia		0.127	**0.019**	
		Birth weight, SDS		−0.062	**0.002**	

Sex and pubertal developmental stage (G/B 1 to 5) were entered as covariates. Skewed variables were logarithmically or square root–transformed before testing. Standardized β-values are reported. Significant P - values are bold.
r^2 is the variance explained by the model.
Abbreviation: SDS, SD score.

obesity and insulin resistance in an effort to increase IS [13, 46]. Consequently, serum irisin concentrations have been found to associate positively with BMI, whole body mass, fat mass and waist-to-hip ratio [47, 48], insulin resistance indices [13, 14, 16, 47], and the risk of metabolic syndrome [13]. However, no [15, 16] or negative [17] correlations between irisin and weight parameters and insulin resistance indices [49] have been reported. We found a negative BMIadj-independent association between irisin and QUICKI. Conflicting results have been reported with regard to serum irisin levels and lipid profile. We found a positive association between irisin and TG, supporting previous data in adults [13, 46] and Korean adolescents [48]. Also, a negative correlation between HDL-C and irisin has been found [13, 16], whereas others have reported no correlation [17, 47], in accordance with our findings. In contrast, two large studies demonstrated irisin to associate with a favorable lipid profile [49, 50]. The

conflicting results could be attributed to the differences in the assays, sample sizes, and physical activity.

In this study, serum irisin had a positive association with GGT independently of BMIadj, supporting the relation between irisin and unfavorable metabolic features. Contradictory results concerning irisin and transaminases have been reported. A negative correlation was found in obese Chinese adults [51], whereas another study demonstrated increased circulating irisin concentrations in patients with metabolic syndrome and fatty liver disease [52]. To our knowledge only one previous study has investigated the relationship between irisin and liver enzymes in prepubertal children; no association between irisin and GGT was found [53].

Li *et al.* [10] found no significant difference in FGF21 concentrations between the sexes before the onset of puberty, whereas after that FGF21 levels declined slightly in boys. However, no effect of sex or puberty have been reported by others [9, 38]. In our study, the slightly higher FGF21 concentration in girls compared with boys did not reach statistical significance. HMW Adn was higher in lean Italian prepubertal [42] and 9- to 10-year-old Japanese girls [43] (without pubertal stage adjustment) compared with boys. In our study of midpubertal children, girls had higher HMW Adn concentrations than boys. Moreover, in boys the decrease in HMW Adn was significant from prepubertal or early pubertal stages toward stage G3, in line with the study of Böttner *et al.* [54] that used total Adn measurements. Furthermore, higher irisin levels have been reported in nonobese girls compared with boys [48], whereas in overweight or obese children or adolescents no sex-related difference has been detected [15, 16, 48]. Reinehr *et al.* [16] reported pubertal children to have significantly higher irisin concentrations compared with prepubertal ones; however, consistent with our findings, pubertal stage [15, 48] or sex [15] had no effect on serum irisin levels in two other studies.

Both low birth weight and preeclampsia predispose to later cardiovascular morbidity [18–20]. In the current study, we found no differences in serum FGF21, HMW Adn, or irisin concentrations or in other measured biochemical parameters between children born SGA, AGA, or from preeclamptic pregnancies. In addition, in linear regression analyses, birth weight or maternal preeclampsia had no association with FGF21 or irisin concentrations, whereas the association between birth weight and HMW Adn was positive. Consistent with our results, low birth weight was associated with lower HMW Adn and increased insulin resistance in obese Mexican children [55], and HMW Adn concentrations tended to fall between 2 and 6 years of age in Catalan SGA-born children with catch-up growth [56]. Although some of the adverse associations between irisin or HMW Adn and some metabolic parameters tended to be stronger in the SGA than AGA subgroup, a more unfavorable metabolic profile with regard to low birth weight or exposure to maternal preeclampsia may be undetectable by the examined parameters.

The strengths of the current study include detailed anthropometric data for the study subjects at birth and at the age of 12 years and numerous biochemical measurements. However, there are also some weaknesses. The study age of 12 years is challenging because of the variable timing in pubertal development. We tried to exclude the possible influence of this variation on our results by several ways. We adjusted the analyses for the pubertal developmental stage. Although we controlled for several confounding factors, the one-time measurement of IS could be a potential limitation in our study. Fasting insulin alone or in combination with fasting glucose is not an optimal measure for assessing individual IS, but they may be applicable in studies with well-defined cohorts [57]. We used QUICKI as a surrogate marker of IS. QUICKI correlates well with the glucose clamp method [58]. Unfortunately, we did not have data on other factors influencing IS, such as dietary habits and the frequency and intensity of exercise. Finally, we measured irisin by using a commercial ELISA kit with a polyclonal antibody [28]. Although the kit used is considered the best validated assay for this molecule to date [12], a more accurate assay to detect serum irisin levels would be desirable.

In conclusion, in this cohort of 12-year-old healthy children, serum irisin predicted reduced IS, although the association was weak. Furthermore, the associations between irisin and IS markers were independent of BMIadj. Serum HMW Adn was a positive predictor for HDL-C, whereas its associations with IS markers were BMIadj dependent. FGF21 had no independent contribution to IS or lipid variables in multivariate regression analyses. Some of the unfavorable associations tended to be stronger in the SGA-born children compared with those born AGA or from preeclamptic pregnancies. This study supports previous findings on the effect of low birth weight on adverse metabolic features, which may remain undetectable by serum irisin, HMW Adn, and FGF21 measurements.

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References and Notes

1. Fasshauer M, Blüher M. Adipokines in health and disease. *Trends Pharmacol Sci*. 2015;**36**(7):461–470.
2. Li F, Li Y, Duan Y, Hu CA, Tang Y, Yin Y. Myokines and adipokines: involvement in the crosstalk between skeletal muscle and adipose tissue. *Cytokine Growth Factor Rev*. 2017;**33**:73–82.
3. Itoh N. FGF21 as a hepatokine, adipokine, and myokine in metabolism and diseases. *Front Endocrinol (Lausanne)*. 2014;**5**:107.
4. Kharitonenkov A, DiMarchi R. Fibroblast growth factor 21 night watch: advances and uncertainties in the field. *J Intern Med*. 2017;**281**(3):233–246.
5. Fisher FM, Kleiner S, Douris N, Fox EC, Mepani RJ, Verdeguer F, Wu J, Kharitonenkov A, Flier JS, Maratos-Flier E, Spiegelman BM. FGF21 regulates PGC-1α and browning of white adipose tissues in adaptive thermogenesis. *Genes Dev*. 2012;**26**(3):271–281.
6. Zhang X, Yeung DC, Karpisek M, Stejskal D, Zhou ZG, Liu F, Wong RL, Chow WS, Tso AW, Lam KS, Xu A. Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans [published correction appears in *Diabetes*. 2019;68(1):235]. *Diabetes*. 2008;**57**(5):1246–1253.
7. Chen C, Cheung BM, Tso AW, Wang Y, Law LS, Ong KL, Wat NM, Xu A, Lam KS. High plasma level of fibroblast growth factor 21 is an Independent predictor of type 2 diabetes: a 5.4-year population-based prospective study in Chinese subjects. *Diabetes Care*. 2011;**34**(9):2113–2115.
8. Bobbert T, Schwarz F, Fischer-Rosinsky A, Pfeiffer AF, Möhlig M, Mai K, Spranger J. Fibroblast growth factor 21 predicts the metabolic syndrome and type 2 diabetes in Caucasians. *Diabetes Care*. 2013;**36**(1): 145–149.
9. Reinehr T, Woelfle J, Wunsch R, Roth CL. Fibroblast growth factor 21 (FGF-21) and its relation to obesity, metabolic syndrome, and nonalcoholic fatty liver in children: a longitudinal analysis. *J Clin Endocrinol Metab*. 2012;**97**(6):2143–2150.
10. Li G, Yin J, Fu J, Li L, Grant SFA, Li C, Li M, Mi J, Li M, Gao S. FGF21 deficiency is associated with childhood obesity, insulin resistance and hypoadiponectinaemia: the BCAMS Study. *Diabetes Metab*. 2017;**43**(3):253–260.
11. Ye R, Scherer PE. Adiponectin, driver or passenger on the road to insulin sensitivity? *Mol Metab*. 2013; **2**(3):133–141.

12. Perakakis N, Triantafyllou GA, Fernández-Real JM, Huh JY, Park KH, Seufert J, Mantzoros CS. Physiology and role of irisin in glucose homeostasis. *Nat Rev Endocrinol*. 2017;**13**(6):324–337.
13. Park KH, Zaichenko L, Brinkoetter M, Thakkar B, Sahin-Efe A, Joung KE, Tsoukas MA, Geladari EV, Huh JY, Dincer F, Davis CR, Crowell JA, Mantzoros CS. Circulating irisin in relation to insulin resistance and the metabolic syndrome. *J Clin Endocrinol Metab*. 2013;**98**(12):4899–4907.
14. Qiu S, Cai X, Yin H, Zügel M, Sun Z, Steinacker JM, Schumann U. Association between circulating irisin and insulin resistance in non-diabetic adults: a meta-analysis. *Metabolism*. 2016;**65**(6): 825–834.
15. Blüher S, Panagiotou G, Petroff D, Markert J, Wagner A, Klemm T, Filippaios A, Keller A, Mantzoros CS. Effects of a 1-year exercise and lifestyle intervention on irisin, adipokines, and inflammatory markers in obese children. *Obesity (Silver Spring)*. 2014;**22**(7):1701–1708.
16. Reinehr T, Elfers C, Lass N, Roth CL. Irisin and its relation to insulin resistance and puberty in obese children: a longitudinal analysis. *J Clin Endocrinol Metab*. 2015;**100**(5):2123–2130.
17. Moreno-Navarrete JM, Ortega F, Serrano M, Guerra E, Pardo G, Tinahones F, Ricart W, Fernández-Real JM. Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. *J Clin Endocrinol Metab*. 2013;**98**(4):E769–E778.
18. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. *Lancet*. 1989;**2**(8663):577–580.
19. Davis EF, Lazdam M, Lewandowski AJ, Worton SA, Kelly B, Kenworthy Y, Adwani S, Wilkinson AR, McCormick K, Sargent I, Redman C, Leeson P. Cardiovascular risk factors in children and young adults born to preeclamptic pregnancies: a systematic review. *Pediatrics*. 2012;**129**(6):e1552–e1561.
20. Stojanovska V, Scherjon SA, Plösch T. Preeclampsia as modulator of offspring health. *Biol Reprod*. 2016;**94**(3):53.
21. Davey DA, MacGillivray I. The classification and definition of the hypertensive disorders of pregnancy. *Am J Obstet Gynecol*. 1988;**158**(4):892–898.
22. Pihkala J, Hakala T, Voutilainen P, Raivio K. [Characteristic of recent fetal growth curves in Finland]. [in Finnish] *Duodecim*. 1989;**105**(18):1540–1546.
23. Tenhola S, Martikainen A, Rahiala E, Herrgård E, Halonen P, Voutilainen R. Serum lipid concentrations and growth characteristics in 12-year-old children born small for gestational age. *Pediatr Res*. 2000;**48**(5):623–628.
24. Rahiala E, Tenhola S, Vanninen E, Herrgård E, Tikanoja T, Martikainen A. Ambulatory blood pressure in 12-year-old children born small for gestational age. *Hypertension*. 2002;**39**(4):909–913.
25. Tenhola S, Rahiala E, Martikainen A, Halonen P, Voutilainen R. Blood pressure, serum lipids, fasting insulin, and adrenal hormones in 12-year-old children born with maternal preeclampsia. *J Clin Endocrinol Metab*. 2003;**88**(3):1217–1222.
26. Tenhola S, Rahiala E, Halonen P, Vanninen E, Voutilainen R. Maternal preeclampsia predicts elevated blood pressure in 12-year-old children: evaluation by ambulatory blood pressure monitoring. *Pediatr Res*. 2006;**59**(2):320–324.
27. Saari A, Sankilampi U, Hannila ML, Kiviniemi V, Kesseli K, Dunkel L. New Finnish growth references for children and adolescents aged 0 to 20 years: length/height-for-age, weight-for-length/height, and body mass index-for-age. *Ann Med*. 2011;**43**(3):235–248.
28. RRID:AB_2783013, http://antibodyregistry.org/search.php?q=AB_2783013.
29. RRID:AB_2783012, http://antibodyregistry.org/search.php?q=AB_2783012.
30. RRID:AB_2783020, http://antibodyregistry.org/search.php?q=AB_2783020.
31. RRID:AB_2783019, http://antibodyregistry.org/search.php?q=AB_2783019.
32. RRID:AB_2783017, http://antibodyregistry.org/search.php?q=AB_2783017.
33. RRID:AB_2783018, http://antibodyregistry.org/search.php?q=AB_2783018.
34. RRID:AB_2783014, http://antibodyregistry.org/search.php?q=AB_2783014.
35. Muniyappa R, Lee S, Chen H, Quon MJ. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. *Am J Physiol Endocrinol Metab*. 2008;**294**(1):E15–E26.
36. Seppä S, Tenhola S, Voutilainen R. Data from: Fibroblast growth factor 21, adiponectin and irisin as markers of unfavorable metabolic features in 12-year-old children. Figshare. Deposited 2018;**02**. doi: 10.6084/m9.figshare.7409531.
37. Ebert T, Gebhardt C, Scholz M, Wohland T, Schleinitz D, Fasshauer M, Blüher M, Stumvoll M, Kovacs P, Tönjes A. Relationship between 12 adipocytokines and distinct components of the metabolic syndrome. *J Clin Endocrinol Metab*. 2018;**103**(3):1015–1023.
38. Korwutthikulrangsri M, Mahachoklertwattana P, Chanprasertyothin S, Pongratanakul S, Poomthavorn P. Serum fibroblast growth factor 21 in overweight and obese Thai children and

adolescents: its relation to glucose metabolism and its change after glucose loading. *Clin Endocrinol (Oxf).* 2015;**83**(6):820–827.
39. Hanks LJ, Casazza K, Ashraf AP, Wallace S, Gutiérrez OM. Fibroblast growth factor-21, body composition, and insulin resistance in pre-pubertal and early pubertal males and females. *Clin Endocrinol (Oxf).* 2015;**82**(4):550–556.
40. Araki S, Dobashi K, Kubo K, Asayama K, Shirahata A. High molecular weight, rather than total, adiponectin levels better reflect metabolic abnormalities associated with childhood obesity. *J Clin Endocrinol Metab*. 2006;**91**(12):5113–5116.
41. Mangge H, Almer G, Haj-Yahya S, Pilz S, Gasser R, Möller R, Horejsi R. Preatherosclerosis and adiponectin subfractions in obese adolescents. *Obesity (Silver Spring).* 2008;**16**(12):2578–2584.
42. Murdolo G, Nowotny B, Celi F, Donati M, Bini V, Papi F, Gornitzka G, Castellani S, Roden M, Falorni A, Herder C, Falorni A. Inflammatory adipokines, high molecular weight adiponectin, and insulin resistance: a population-based survey in prepubertal schoolchildren. *PLoS One.* 2011;**6**(2):e17264.
43. Ochiai H, Shirasawa T, Nishimura R, Nanri H, Ohtsu T, Hoshino H, Tajima N, Kokaze A. Abdominal obesity and serum adiponectin complexes among population-based elementary school children in Japan: a cross-sectional study. *BMC Pediatr*. 2014;**14**:81.
44. McCourt HJ, Hunter SJ, Cardwell CR, Young IS, Murray LJ, Boreham CA, McEneny J, Woodside JV, McKinley MC. Adiponectin multimers, body weight and markers of cardiovascular risk in adolescence: Northern Ireland Young Hearts Project. *Int J Obes.* 2013;**37**(9):1247–1253.
45. Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Boström EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S, Højlund K, Gygi SP, Spiegelman BMA. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature*. 2012;**481**(7382):463–468.
46. Crujeiras AB, Pardo M, Casanueva FF. Irisin: "fat" or artefact. *Clin Endocrinol (Oxf).* 2015;**82**(4): 467–474.
47. Sesti G, Andreozzi F, Fiorentino TV, Mannino GC, Sciacqua A, Marini MA, Perticone F. High circulating irisin levels are associated with insulin resistance and vascular atherosclerosis in a cohort of nondiabetic adult subjects. *Acta Diabetol.* 2014;**51**(5):705–713.
48. Jang HB, Kim HJ, Kang JH, Park SI, Park KH, Lee HJ. Association of circulating irisin levels with metabolic and metabolite profiles of Korean adolescents. *Metabolism*. 2017;**73**:100–108.
49. Buscemi S, Corleo D, Vasto S, Buscemi C, Massenti MF, Nuzzo D, Lucisano G, Barile AM, Rosafio G, Maniaci V, Giordano C. Factors associated with circulating concentrations of irisin in the general population cohort of the ABCD study. *Int J Obes.* 2018;**42**(3):398–404.
50. Oelmann S, Nauck M, Völzke H, Bahls M, Friedrich N. Circulating irisin concentrations are associated with a favourable lipid profile in the general population. *PLoS One.* 2016;**11**(4):e0154319.
51. Zhang HJ, Zhang XF, Ma ZM, Pan LL, Chen Z, Han HW, Han CK, Zhuang XJ, Lu Y, Li XJ, Yang SY, Li XY. Irisin is inversely associated with intrahepatic triglyceride contents in obese adults. *J Hepatol.* 2013;**59**(3):557–562.
52. Choi ES, Kim MK, Song MK, Kim JM, Kim ES, Chung WJ, Park KS, Cho KB, Hwang JS, Jang BK. Association between serum irisin levels and non-alcoholic fatty liver disease in health screen examinees. *PLoS One.* 2014;**9**(10):e110680.
53. Viitasalo A, Atalay M, Pihlajamäki J, Jääskeläinen J, Korkmaz A, Kaminska D, Lindi V, Lakka TA. The 148 M allele of the PNPLA3 is associated with plasma irisin levels in a population sample of Caucasian children: the PANIC Study. *Metabolism*. 2015;**64**(7):793–796.
54. Böttner A, Kratzsch J, Müller G, Kapellen TM, Blüher S, Keller E, Blüher M, Kiess W. Gender differences of adiponectin levels develop during the progression of puberty and are related to serum androgen levels. *J Clin Endocrinol Metab*. 2004;**89**(8):4053–4061.
55. Domínguez Hernández C, Klünder M, Huang F, Flores Armas EM, Velázquez-López L, Medina-Bravo P. Association between abdominal fat distribution, adipocytokines and metabolic alterations in obese low-birth-weight children. *Pediatr Obes.* 2016;**11**(4):285–291.
56. Ibáñez L, Lopez-Bermejo A, Diaz M, Angulo M, Sebastiani G, de Zegher F. High-molecular-weight adiponectin in children born small- or appropriate-for-gestational-age. *J Pediatr*. 2009;**155**(5):740–742.
57. Levy-Marchal C, Arslanian S, Cutfield W, Sinaiko A, Druet C, Marcovecchio ML, Chiarelli F; ESPE-LWPES-ISPAD-APPES-APEG-SLEP-JSPE; Insulin Resistance in Children Consensus Conference Group. Insulin resistance in children: consensus, perspective, and future directions. *J Clin Endocrinol Metab*. 2010;**95**(12):5189–5198.
58. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab*. 2000;**85**(7):2402–2410.